The B analyses were made by J. Thoburn; the C, H and N by H. Beck and V. Stryker.

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RECEIVED JUNE 23, 1954

THE DEGRADATION OF CHOLESTEROL BY MAMMALIAN TISSUE EXTRACTS

Sir:

As part of a study concerned with the metabolic degradation of cholesterol and its conversion to bile acids, we have successfully prepared aqueous particle-free extracts of mammalian tissue which are capable of degrading the side-chain of cholesterol. The major product, obtained in yields up to 5%, has been isolated and identified as isocaproic acid. Active enzyme preparations have been obtained from beef adrenals, ovary, testis and rat liver. Extracts of the first three tissues were obtained by homogenization in 0.3 *M* sucrose followed by high speed centrifugation (85,000 *G*) at 0° for 30 minutes to remove the particulate fraction. The sedimented material was essentially inactive. The aqueous phase required only adenosine triphosphate and diphosphopyridine nucleotide for activity. This was demonstrated by precipitation of the active enzymes by half saturation with $(NH_4)_2SO_4$, followed by dialysis of the precipitate for 24 hours against cold water. The resulting solution, which was rendered inactive by stirring for a few minutes with charcoal, was restored to activity by the addition of the above cofactors. In most instances the enzyme preparations were incubated for three hours at 37° under oxygen or air at a *p*H of 8.3 (0.07 *M* tris-(hydroxymethyl)-aminomethane buffer). The substrate was an albumin stabilized emulsion[1] of cholesterol-26-C^{14}.[2]

The radioactive isocaproic acid was isolated by steam distillation of the acidified extracts followed by paper chromatography[3] (diethylamine–butanol; R_f 0.67). After elution from the papers and subsequent addition of carrier isocaproic acid, the anilide (m.p. 110°) and the *p*-bromophenacyl ester (m.p. 76–77°) were prepared. The specific activity of these derivatives remained constant despite repeated recrystallizations. Although small amounts of radioactivity could be detected in other acids, the isocaproic acid invariably contained the greatest radioactivity.

Following incubation of the adrenal extracts with cholesterol-4-C^{14} [4] several radioactive steroids have been isolated by standard chromatographic procedures.[5] Surprisingly, neither progesterone nor pregnenolone were found to be radioactive; negative results were obtained regardless of whether these two steroids were added as carrier prior to or following incubation.

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(2) W. G. Dauben and H. L. Bradlow, *ibid.*, **72**, 4248 (1950).

(3) A. R. Jones, E. J. Dowling and W. J. Skraba, *Anal. Chem.*, **25**, 394 (1953).

(4) R. B. Turner, THIS JOURNAL, **72**, 579 (1950); R. D. H. Heard and P. Ziegler, *ibid.*, **73**, 4036 (1951); W. G. Dauben and J. F. Eastham, *ibid.*, **73**, 4463 (1951).

(5) A. Zaffaroni and R. B. Burton, *J. Biol. Chem.*, **193**, 749 (1951); R. Neher and A. Wettstein, *Helv. Chim. Acta*, **35**, 276 (1952).

The active system in rat liver was found to be localized in the particulate fraction which could be extracted by stirring with water to obtain a soluble preparation. In this tissue the original cytoplasmic supernatant fluid was found to be inhibitory unless the proteins were denatured by boiling and subsequently removed by centrifugation.

It is hoped that a more extensive report of this study will be published shortly.

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RECEIVED JUNE 24, 1954

THE ACONITE ALKALOIDS. XXVI. OXONITINE AND OXOACONITINE

Sir:

Permanganate oxidation of aconitine,[1] $C_{34}H_{47}NO_{11}$, furnishes two neutral products, oxonitine[2,3,4] and oxoaconitine.[3,4] The nature and formulation of the former has long been in doubt.[2,4] However, the formula $C_{32}H_{41}NO_{12}$ based in part on an oxidative scission of the N-ethyl group is still accepted by some.[1,5] Because of the important relationship these substances bear to a correct interpretation of the structure of aconitine, we wish to present evidence showing that oxidation of aconitine to oxonitine as well as to oxoaconitine *proceeds without the loss of carbon atoms.*

It appears that the use of acetic acid or chloroform to recrystallize oxonitine has led to incorrect formulations due to retention of solvent. When recrystallized from ethanol, benzene or acetone, the data obtained clearly support a $C_{34}H_{45}NO_{12}$ formulation. Calcd. for $C_{34}H_{45}NO_{12}$: C, 61.90; H, 6.87. Found: (EtOH) C, 61.73, 61.66; H, 6.65, 6.86; (benzene) C, 61.83, 61.81; H, 6.74, 6.90; (acetone) C, 61.88, 62.10; H, 6.76, 6.73; (pyridine–acetone) C, 62.08; H, 6.64. Oxonitine from $CHCl_3$ or CH_2Cl_2 gave consistently low carbon values and showed the presence of several per cent. chlorine. Our oxonitine melted at 279–284° though occasionally 288–293° was noted apparently due to dimorphism. $[\alpha]^{27}D$ −49° (*c* 0.25 in chf.). Oxonitine in contrast to oxoaconitine did not form an oxime.

The formulation of oxoaconitine has now been revised to $C_{34}H_{43}NO_{12}$; m.p. 266–272.5°, $[\alpha]^{27}D$ −100° (*c* 0.3 in chf.). Calcd. for $C_{34}H_{43}NO_{12}$: C, 62.09; H, 6.59. Found: C, 61.90; H, 6.57. Oxime, m.p. 282–285.5°. Calcd. for $C_{34}H_{44}N_2O_{12}$; C, 60.70; H, 6.59, N, 4.17. Found: C, 60.59; H, 6.67; N, 4.37.

The formation of oxonitine and oxoaconitine from aconitine appears to involve oxidation of an N-ethyl group to N-acetyl. Their behavior toward

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(2) F. H. Carr, *J. Chem. Soc.*, **101**, 2241 (1912); E. Späth and F. Galinovsky, *Ber.*, **63**, 2994 (1930); A. Lawson, *J. Chem. Soc.*, 80 (1936); R. Majima and K. Tamura, *Ann.*, **526**, 116 (1936).

(3) W. A. Jacobs and R. C. Elderfield, THIS JOURNAL, **58**, 1059 (1936).

(4) W. A. Jacobs, R. C. Elderfield and L. C. Craig, *J. Biol. Chem.*, **128**, 439 (1939).

(5) O. E. Edwards and L. Marion, *Canad. J. Chem.*, **30**, 627 (1952).

acid suggests the presence of a hindered N-acetyl group (barring rearrangement). Thus oxonitine with methanolic · HCl yielded a basic and a neutral fraction. From the latter was isolated a substance (I) which results from the replacement of the acetoxy group by a methoxyl group; m.p. 265–268°, $[\alpha]^{29}D$ −49° (*c* 0.47 in chf.). Calcd. for $C_{33}H_{45}NO_{11}$: C, 62.74; H, 7.18; OCH_3, 24.56. Found: C, 62.84, 62.70; H, 7.46, 7.09; OCH_3, 24.07, 23.91. On saponification 16.115 mg. consumed 0.21 ml. of 0.1 *N* NaOH; calcd. for 1 equiv., 0.25 ml.

The basic fraction yielded a secondary base (II)[4] formed by the loss of the N-acetyl group from I, m.p. 248–249°, $[\alpha]^{29}D$ +33° (*c* 0.5 in chf.) Calcd. for $C_{31}H_{43}NO_{10}$: C, 63.14; H, 7.35; N, 2.38. Found: C, 62.87; H, 7.28, N. 2.61. Its N-nitroso derivative formed microplatelets, m.p. 246–248.5°. Calcd. for $C_{31}H_{42}N_2O_{11}$: C, 60.18; H, 6.84; N, 4.53. Found: C, 60.18, H, 6.78, N, 4.50. 15.175 mg. on saponification consumed 0.228 ml. of 0.1 *N* NaOH; calcd. for 1 equiv., 0.249 ml.

Treatment of I with Ac_2O gave a mixture from which was isolated a compound (III) containing an extra O-acetyl group, m.p. 290–296°, $[\alpha]^{29}D$ −56° (*c* 0.18 in chf.). Calcd. for $C_{35}H_{47}NO_{12}$: C, 62.39; H, 7.03. Found: C, 62.11; H, 6.92. A substance indistinguishable from III was obtained by acetylation of base II, m.p. 291–297°, $[\alpha]^{30}D$ −56° (*c* 0.5 in chf.). No m.p. depression was observed. Found: C, 62.20; H, 7.00.

Treatment of oxoaconitine with methanolic HCl also yielded basic and neutral fractions. From the latter a substance (IV) was isolated which results from loss of a mole of methanol but with retention of the acetoxy group, m.p. 271–273.5°, $[\alpha]^{28}D$ +22° (*c* 1.03 in chf.). Calcd. for $C_{33}H_{39}NO_{11}$: C, 63.35; H, 6.28; OCH_3, 14.88. Found: C, 63.24; H, 6.17; OCH_3, 14.59.

From the basic fraction a secondary base (V) melting at 180–185° was obtained, $[\alpha]^{28}D$ + 69° (*c* 0.51 in chf.). Calcd. for $C_{31}H_{37}NO_{10}$: C, 63.79; H, 6.39; OCH_3, 15.95. Found: C, 63.55; H, 6.27; OCH_3, 16.34. Its N-nitroso derivative formed minute prisms from dilute acetone, m.p. 272–275.5°. Calcd. for $C_{31}H_{36}N_2O_{11}$: C, 60.77; H, 5.92; OCH_3, 15.20. Found: C, 60.78; H, 5.91; OCH_3, 15.18. V was also obtained by treatment of IV with methanolic HCl, m.p. 179–185°. Found: C, 63.41; H, 6.44. Acetylation of either IV or V gave mixtures from which identical N-acetyl derivatives (VI) were isolated. VI contained an additional O-acetyl group. VI obtained from IV had m.p. 274–279°, $[\alpha]^{29}D$ +9.4° (*c* 0.32 in chf.). Calcd. for $C_{35}H_{41}NO_{12}$: C, 62.96; H, 6.19. Found: C, 63.42; H, 6.26. VI obtained from V had m.p. 272–277°, $[\alpha]^{29}D$ +9.7° (*c* 0.31 in chf.). Found: C, 63.07; H, 6.24. A mixture of the two derivatives showed no m.p. depression.

The origin and nature of the neutral nitronitroso derivative,[6] $C_{31}H_{35}N_3O_{13}$, obtained by the action of nitric acid on oxonitine and oxoaconitine can now be interpreted more readily. It apparently is formed from oxoaconitine by the loss of a mole of methanol, by replacement of the N-acetyl group with a nitroso group and by the introduction of a nitro group. In the case of oxonitine, a preliminary oxidation of CHOH to C=O must occur. The details of these transformations will be presented more fully at a later date.

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RECEIVED JUNE 21, 1954

THE INFLUENCE OF HYDROGEN AND CARBON MONOXIDE PARTIAL PRESSURES ON THE RATE OF THE HYDROFORMYLATION REACTION

Sir:

Previous work[1] in this laboratory on the kinetics of the hydroformylation reaction showed that the rate was proportional to the concentration of olefin and approximately proportional to the concentration of dicobalt octacarbonyl catalyst, while it was essentially independent of the pressure of synthesis gas (H_2:CO, 1–2) in the range of 120 to 380 atm. Since the reaction is thermodynamically possible at low pressures of synthesis gas,[2] we have now studied the hydroformylation reaction at low partial pressures of hydrogen and carbon monoxide.

Contrary to the prevailing opinion that increased pressure facilitates the hydroformylation reaction, our new semi-quantitative data secured with cyclohexene at 110 and 115° under widely differing H_2:CO partial pressures show the following surprising results: (1) At constant carbon monoxide pressures the rate greatly increases with increasing hydrogen pressure. (2) At constant hydrogen partial pressures, the rate increases with increasing partial pressures of carbon monoxide up to about 10 atm. but decreases with higher partial pressure of carbon monoxide.

It is thus apparent that our previous observation on the independence of rate with pressure was owing to the relatively equal but opposite effects of increasing the partial pressure of the two gases, hydrogen and carbon monoxide.

Experimental.—A solution of cyclohexene and dicobalt octacarbonyl (34 g. and 1.4 g. per 100 g. of solution, respectively) in toluene was treated at 110 ± 1° for 68 minutes in a series of experiments under 10 atm. of CO and at various partial pressures of H_2. When the partial pressure of H_2 was 27, 54 and 110 atm., the conversion of olefin to cyclohexanecarboxaldehyde was 30, 51, and 65%, respectively.

With 55 atm. of H_2 and under otherwise identical conditions except that the partial pressure of CO was 3, 5, 14, 28 and 54 atm., olefin conversions of 35, 46, 43, 29 and 18%, respectively, were secured.

With a constant H_2:CO ratio of unity and total pressures of 53, 110 and 220 atm., the conversion of olefin was 15.3, 18 and 17.3%, respectively, again illustrating the apparent independence of rate at relatively high pressure of 1:1 gas.

When 2-ethyl-1-hexene was employed, essentially the same results as above were secured.

Discussion.—The above data may be explained if, in the initial stage of the reaction, some equilib-

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